changing external conditions. It also makes it possible to identify optimal solutions to the adaptation problem, which can be experimentally validated. Under optimal conditions the intrinsically non-linear dynamics of these networks becomes simple and linear with respect to the components of the proteome, while the complex dynamics of the concentrations of the signal molecules requires non-linear mathematics: that what is controlled obeys very simple dynamics while the control systems remain non-linear and complex. This allows for simple, analytical solutions to previously intractable dynamic problems. We are therefore optimistic that the present work, focused on the fitness value of bacterial populations, will contribute to quantitative integration of bacterial physiology and population genetics.

1054-Symp
How Initiation Factors Regulate the Subunit Joining Step of Translation Initiation
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High-fidelity initiator transfer RNA (tRNA) and messenger RNA (mRNA) start codon selection during translation initiation is essential for ensuring the integrity of gene expression; mis-initiation using an elongator tRNA or a sense codon can lead to proteins containing aberrant N-termini or to errors in reading frame selection that yield truncated and/or mis-folded proteins. In bacteria, three essential initiation factors (IFs) bind to the small, 30S, ribosomal subunit and control the accuracy of translation initiation by regulating tRNA and codon selection into the 30S initiation complex (30S IC) and joining of the large, 50S, ribosomal subunit to the 30S IC to form an elongation-competent 70S initiation complex (70S IC). Although the IFs render the rate of 50S subunit joining to the 30S IC dependent on the presence of a correctly selected initiator tRNA that is basepaired to a start codon within the 30S IC, the mechanism through which the IFs accomplish this remains elusive. Using fluorescently labeled IFs, tRNAs, and ribosomal subunits, we have recently developed several single-molecule fluorescence resonance energy transfer signals that report on the conformational dynamics of the 30S IC and on the dynamics of 50S subunit joining to the 30S IC. Here we will present the results of our latest studies, which, when integrated with the available biochemical data, strongly suggest that tRNA- and codon-dependent conformational dynamics of the IFs play a critical role in regulating the 50S subunit joining step of translation initiation.

1055-Symp
Snapshots of the Ribosomal Machinery by Cryo-Electron Mycroscopy
Roland Beckmann.
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Protein synthesis in all cells is performed by ribosomes, large protein-RNA complexes. Ribosomes undergo substantial conformational changes during their activity cycle and engage in interactions with numerous additional factors. In order to provide a mechanistic understanding of the underlying processes we aim at providing structural information on these complexes at highest possible resolution. We combine in vitro reconstitution and biochemical methods with cryo-electron microscopy and single particle analysis for structure determination. Furthermore complementary methods such as small angle X-ray scattering (SAXS), X-ray crystallography or Molecular Dynamics based simulations can be employed that often allow interpretations at the molecular level. Here we present snapshots of the ribosomal machinery engaged in several cotranslational events such as cotranslational protein folding, membrane translocation or insertion via the Sec complex.

1056-Symp
Observation of MRNA Surveillance in Living Yeast by Ribosome Profiling
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The synthesis of proteins is a major step in gene expression and therefore represents a regulatory point for modulating cellular output. During elongation, ribosomes are thought to pause along the mRNA transcript upon colliding with various obstacles or translating specific sequence motifs. In addition, ribosomes halt if they arrive at the 3' end of a transcript after failing to properly terminate or if the message is endonucleolytically cleaved. Genetic studies have suggested that the protein Dom34 and a GTPase binding partner, Hbs1, target mRNAs associated with paused ribosomes for degradation (Doma and Parker 2006, Nature 440:461-4). While biochemical data support this idea by showing that Dom34 directly binds and dissociates ribosomes (Shoemaker et al. 2010, Science 330:369-72), the natural cellular targets of Dom34 remain unknown. To identify functions of Dom34 in vivo, knockout and wild type strains of S. cerevisiae were subjected to a high-throughput footprinting analysis (ribosome profiling) that reveals the places where ribosomes are bound throughout the transcriptome. These data reveal that ribosome occupancy on known pauses is not generally enhanced in the strain lacking Dom34. Instead, the targets of Dom34 include a small number of specialized targets in open reading frames. In addition, ribosomes are enriched at the 3' end of many transcripts in the Dom34 deletion strain and generally covere <10 bp of polyadenine at the 3' end of the protected fragment. These data imply that Dom34 dissociates ribosomes that bypass the stop codon and thus serves as a quality control factor required for recycling ribosomes that fail to terminate by the conventional pathway (mediated by eRF1, eRF3, and Rli1). These results support a general role for Dom34 in ribosome recycling in addition to a more specialized role on particular gene products.

Symposium: Frontiers in Electron Miscroscopy

1057-Symp
Electron-Cryomicroscopy: From Molecules to Cells
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Cryoelectron microscopy makes possible structural studies of molecules or cells under close-to-life conditions. There are three major imaging modalities: electron crystallography, single particle analysis and electron tomography. Electron crystallography requires the molecules under scrutiny to form two-dimensional crystals, single particle analysis can deal with individual macromolecules or molecular assemblies, while electron tomography allows studying molecular structures in situ, i.e. in their functional cellular environment. The 26 S proteasome, a complex of 2.5 MDa, will be used to exemplify the potential of single particle analysis. EM density maps at subnanometer resolution of this complex in different nucleotide-bound states are interpreted beyond their nominal resolution by the integration of orthogonal dates. Several examples will be used to demonstrate the power of electron tomography for in situ structural studies of very large and labile supramolecular assemblies.
Beck, F., Unverdorben, P., Bohn, S., Schweitzer, A., Pfeifer, G., Sakata, E., Nickell, S., Plitzko, J.M., Villa, E., Baumeister, W., Frster, F.: Near-atomic resolution structural model of the yeast 26S proteasome, P. Natl. Acad. Sci. Vol. 109, no. 37 (2012). Brandt, F., S.A. Etchells, J.O. Ortiz, A.H. Elcock, F.U. Hartl and W. Baumeister: The native 3D organization of bacterial polysomes. Cell 136, 261-271 (2009). Brandt, F., L.-A. Carlson, F.U. Hartl, W. Baumeister and K. Grnewald: The three-dimensional organization of polyribosomes in intact human cells. Mol. Cell 39, 560-569 (2010).

1058-Symp
Two Case Studies where Electron Cryotomography has Revealed Mechanism: ESCRT and the Type VI Secretion System
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Sometimes all that is really needed to distinguish between competing models is a few really good pictures. Electron cryotomography has increasingly met that need by delivering unprecedented 3-D images of intact cells in a near-native (frozen-hydrated) state to ''macromolecular'' resolution. I will present two case studies. In the first, cryotomograms of dividing Sulfolobus acidocaldarius cells revealed a thin layer of material at the leading edge of ingressing membranes whose structure and development point uniquely to an ''hourglass'' model for how the ESCRT machinery drives membrane scission. In the second, cryotomograms of Vibrio cholerae cells revealed extended cytoplasmic tubes in two different conformational states. Identification of the tubes as Type VI Secretion Systems led immediately to a ''spring-loaded dagger'' model for their ability to deposit toxins into neighboring cells.

1059-Symp
Cryoem Studies of Membrane-Protein Interactions
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The processes of membrane fission and fusion both involve an intermediate state called hemi-fission or hemi-fusion, respectively. This intermediate is fundamental and controls the outcome of both processes. During hemi-fusion/fission, the outer leaflet of a budding vesicle is still connected to the plasma membrane, while the inner leaflet has gone through separation/fission. It is particularly critical for fast-paced endocytosis in neural synapses. At the budding neck, this intermediate assumes the form of a tubular micelle. We investigate the interactions of neural synaptic proteins alpha-synuclein and endophilin with lipid vesicles and their ability of membrane remodeling in vitro by cryo-EM, and found that both proteins are capable of inducing the formation of tubular micelles.